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Modelling Skeletal Muscle Motor Unit Recruitment Contributions To Contractile Function: Part 2 - Total (aerobic + anaerobic) ATP Turnover

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Abstract

The purpose of this research was to expand understanding of the energetics of skeletal muscle contractions through modeling the total ATP turnover ($totATP_{to}$ = aerobic + anaerobic) of different muscle fibre types and motor units. Contraction conditions involved varied motor unit recruitment for a single contraction, in addition to 3 min of repeated contractions across varying contraction frequencies (0.5 to 2.0 Hz in 0.5 Hz increments) and fractional motor unit recruitment (0.5 to 0.95 in 0.05 increments). Contractile power data was obtained from prior research of the vastus lateralis and converted to $totATP_{to}$ from the free energy release of ATP hydrolysis (50 $kJ \times M^{-1}$) and the cellular efficiency of free energy transfer from ATP hydrolysis during muscle contraction (40%). A computational model was then developed for $totATP_{to}$ for repeated contractions spanning 0.5 to 2.5 Hz and fractional recruitment of 0.5 to 0.9 across 4 different genetic expressions of slow twitch (ST; Type I and I - IIa) to fast twitch (FT; Type IIa, IIab, IIb) proportionality. Completion of 3 min of repeated contractions for 0.4, 0.65 and 0.9 fractional recruitment for the 2 extremes of motor unit proportions (%ST - FT = 80 - 20 to 20 - 80) for contractions at 1 and 2 Hz resulted in $totATP_{to}$ of 30.28 vs. 55.66 vs. 110.28 $mmol \cdot L^{-1}$ (0.4, 0.65, 0.9; 80 – 20; 1 Hz); 50.24 vs. 130.51 vs. 275.58 $mmol \cdot L^{-1}$ (0.4, 0.65, 0.9; 20 – 80; 1 Hz); 60.57 vs. 111.31 vs. 220.55 $mmol \cdot L^{-1}$ (0.4, 0.65, 0.9; 80 – 20; 2 Hz); 100.49 vs. 261.03 vs. 551.16 $mmol \cdot L^{-1}$ (0.4, 0.65, 0.9; 20 – 80; 2 Hz). The 0.65 fractional recruitment data most reflect prior in-vivo whole muscle research of $totATP_{to}$ data and reveal the construct validity of the model. Further modelling is needed to improve our understanding of the metabolic significance of motor unit recruitment to skeletal muscle cellular energy transfer during exercise.

Keywords

Motor unit recruitment; Muscle fibre types; Skeletal muscle; Contractile power; Adenosine triphosphate (ATP); ATP turnover (ATP_{to})

Introduction

Adenosine triphosphate (ATP) serves as the primary cellular energy currency, fueling countless biological processes that sustain life, such as skeletal muscle contraction, protein synthesis, substrate phosphorylation and nerve impulse transmission. Historically, skeletal muscle ATP turnover (ATP_{to}) was dependent upon muscle biopsy samples and the anaerobic component ($anATP_{to}$) has been indirectly calculated from the accumulation of glycolytic intermediates and lactate (1-3). Currently, no *in-vivo* method exists for the measurement of aerobic ATP_{to} for single muscles or muscle fibres. Methods also exist for calculating ATP_{to} from phosphorous magnetic resonance spectroscopy (^{31}P MRS) (4), however, as with the biopsy method both sample a mixture of muscle fibres from different motor units with no control over their proportionality within the sample or for whether these muscle fibres were or were not from recruited motor units.

Obtaining samples from whole muscle is problematic because there are extreme differences in the metabolic capacities and contractile power of the different muscle fibre types (5,6), in addition to variability in their spatial distribution within a muscle (7-9). In short, mixed muscle fibre sampling provides an average of an unknown fibre composition and constrains understanding of the complexities of discrete cellular contributions of the different muscle fibre types to contractile power and the metabolic pathways that support it.

A summary of the results of prior research of human muscle $anATP_{to}$ during intense exercise is presented in Figure 1. In each of these studies, subjects exercised to volitional exhaustion, or for Spriet et al. (3) were exposed to artificial electrical stimulation to contractile failure (less than 20% of initial contractile force). Spriet et al. (3) quantified muscle $anATP_{to}$ in 7 subjects using an invasive protocol of intense exercise to contractile failure through artificial stimulation of the quadriceps femoris muscles located in the anterolateral aspect of the thigh. Muscle biopsies were obtained from the vastus lateralis (VL) after 16, 32, 48, and 64 contractions, and muscle specimens were freeze-dried, removed of their blood and connective tissue, frozen and later analyzed for ATP, ADP, PCr, Cr and lactate. This was completed in both legs of the participants, and the results were the sum of the measurements based on mathematical adjustments to muscle lactate accumulation (Equation 1). This study did not investigate or calculate data for specific motor unit types. Therefore, these results were once again in adherence to the "black box" model of muscle biochemical research, with the resulting mixed muscle anaerobic $anATP_{to}$ equaling 88.35 $mmol \cdot L^{-1}$ muscle water.

Each of Bangsbo et al. (1) and Medbo and Tabata (2) used intense cycle ergometry exercise and muscle biopsy sampling in their methodologies. Bangsbo et al. (1) studied 8 healthy, male subjects during one-

legged, supine, dynamic, knee extensor exercise (52 - 79 Watts) on a modified cycle ergometer to volitional exhaustion (2.2 - 4.9 min). Muscle biopsies were obtained immediately post-exercise from the VL and as with Spriet et al. (3) muscle $anATP_{to}$ was calculated based on mathematical adjustment of muscle lactate accumulation (Equation 2).

$$\mathrm{ATP_{to}} = \frac{\left((1.5 \times \Delta[\mathrm{La}\ -]) + \Delta[\mathrm{CrP}] + (2 \times \Delta[\mathrm{ADP}]) - \Delta[\mathrm{ADP}]\right)}{\mathrm{Time\ (s)}} \qquad Equation\ 1$$

$$\mathrm{ATP_{to}} = \frac{(1.5 \times \Delta[\mathrm{La}\ -] + \Delta[\mathrm{CrP}] + \Delta[\mathrm{ATP}])}{\mathrm{Time\ (s)}} \qquad Equation\ 2$$

Results revealed that the muscle [ATP] decreased by 30% during intense exercise from its original resting concentration of 6·2 $mmol \times kg^{-1}$ wet wt., with added ATP release from metabolism resulting in an $anATP_{to}$ of 109.34 $mmol \times L^{-1}$ muscle water. Medbo and Tabata (2) studied 16 healthy, young, male subjects during bouts of cycle ergometry at intensities pre-determined to cause volitional exhaustion in either of 30 seconds, 1 minute, or 2 - 3 min, with this last longer duration condition being the trial of interest (intensity = 4.8 $Watts \cdot kg^{-1}$; exercise duration = 155 s). Muscle biopsies were obtained from the VL immediately post-exercise, with biopsies occurring from both legs, and where muscle $anATP_{to}$ was calculated as per the computational methods of Bangsbo et al. (1) (Equation 2).

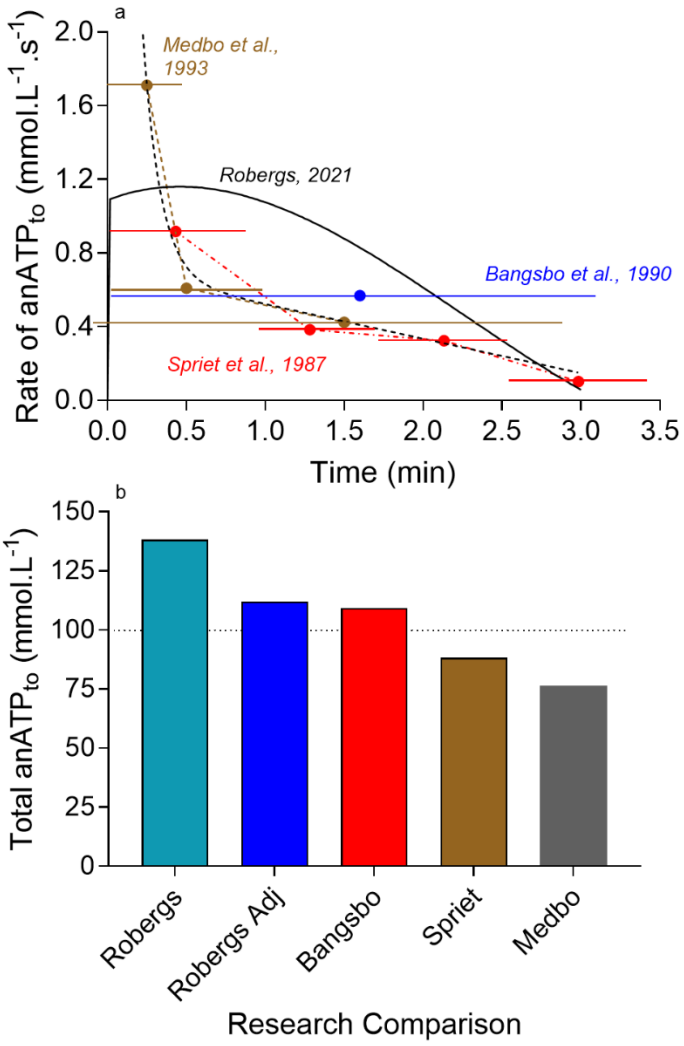

Figure 1. (a) Comparison of data for the rate of anaerobic ATP turnover ($anATP_{to}$) from Robergs (12), Bangsbo et al. (1), Spriet et al. (3), and Medbo and Tabata (2). Horizontal lines represent the time duration of obtaining the muscle samples. (b) The results for calculated total anATP turnover from the respective studies. The figure was adapted from Robergs (11,12). Note, the data for Robergs Adj represents a correction of the total anATPto from the prior modelled data based on the ATP involvement in the PFK reaction (subtracted from the larger total), which was an included errant computational feature of the results from all other studies. See Robergs (12) for detailed explanations.

There is an inability to sustain high $anATP_{to}$ during intense exercise (highlighted in Figure 1a). This is due to the metabolic determinants of muscle contractile failure, which while multifaceted, is largely determined by the rapid increase in intramuscular inorganic phosphate (Pi) due to the increasing imbalance between cytosolic ATP regeneration and mitochondrial respiration. The accumulation of Pi ionically interacts with calcium and interferes with the effectiveness of calcium regulation of muscle contraction. Hence, the decrease in contractile power and the related decrease in $anATP_{to}$ (10).

Robergs (11) and Robergs (12) modelled skeletal muscle $anATP_{to}$ from the VL based on calculations from prior research of muscle metabolite accumulation and related substrate flux for reactions of glycolysis and the phosphagen system. The modelled exercise condition consisted of 1.5 min (11) and 3 min (12) of intense cycle exercise to contractile failure. It is logical that their data for ATP_{to} is larger than that of the muscle biopsy research studies, simply because the biopsy procedure samples a mixture of random muscle fibres across multiple but unknown motor unit categories and could even include fibres from non-recruited motor units. There is also concern over the calculations made from accumulated muscle lactate concentrations based on the unlikely scenario of all substrate flux through glycolysis ending in lactate production and that the 1.5 constant in Equations 1 and 2 has an adjustment for the ATP cost of the PFK reaction of phase I of glycolysis, which contributes to ATP_{to} and theoretically the adjustment lowers gross glycolytic total ATP_{to} by 25%.

Although prior research completed calculations of muscle $anATP_{to}$ from muscle metabolite accumulation, this is not the only indirect method that is suitable. Cellular bioenergetics adheres to the laws of thermodynamics, where knowing muscle contractile power, cellular metabolic energy transfer efficiency and the in-vivo energy transfer of ATP hydrolysis enables the calculation of cellular total ATP_{to} ($totATP_{to}$) and as such the values for $totATP_{to}$ will be much larger than $anATP_{to}$. Such procedures are explained in Methods.

Statement of The Problem and Purpose

Current teaching and research of topics within neuromuscular physiology continue to apply a model of muscle contraction that ignores the complexities of differences in contractile force and power and metabolic capacities between the different muscle fibre types. As we are currently unable to measure changes in motor unit recruitment in humans, or in animal models, the development of models that can extrapolate current research findings of the contractile and metabolic characteristics of skeletal muscle fibres remains the only method to further understanding of this subject.

Consequently, the purpose of this research was to extrapolate prior measured and modelled data for skeletal muscle single fibre contractions through calculation adjustments based on the cellular biochemical efficiency of muscle contraction, resulting in estimates for cellular $totATP_{to}$. As the developed model for motor unit recruitment included 5 different muscle fibres/motor unit types, new knowledge will be gained on how sequential motor unit recruitment governs the contribution of the different motor unit types to the $totATP_{to}$ of muscle contraction during different intensities and durations (repeated contractions at different

rates of contraction). Results from this study may inform robotics effort to mimic human movement mechanics.

Methods

Due to the inability to directly measure the incremental influence of motor unit recruitment to whole muscle contractile velocity, force, power and the resulting $totATP_{to}$, there is logic and scientific rationale for the development of a computational model to direct inquiry into this topic. As such, this study employed both quantitative and computational methodology.

Prior research from this team developed a model of incremental motor unit recruitment to calculate individual motor unit contributions to the contractile velocity, force, and power of the VL (13). This prior research was based on data of single muscle fibre contractile force, velocity and power from multiple research groups (5,6), and used with assumed motor unit sizes and different genetic proportions of motor unit expressions of five different fibre (motor unit) types to calculate (model) incremental motor unit recruitment influences to contractile force, velocity and power. The resulting data for the motor unit contributions to muscle contractile power was retrieved from this research and utilized in the development of an additional model for the computation of $totATP_{to}$, which then resulted in this manuscript.

To ascertain the prior research of skeletal muscle $totATP_{to}$ during exercise, literature searches were completed using a combination of PubMed, ScienceDirect and EBSCOhost based on search terms consisting of skeletal muscle, exercise, and ATP_{to}. As quality research on muscle biochemistry dates to the 1970s, database searches were not date constrained. The prior model of sequential motor unit recruitment was expanded to allow the computation of muscle ATP_{to} and details of the added model development are summarized below.

Modelling Fibre Type and Motor Unit Total ATP_{to}

The data for contractile power of the VL resulting from each additional increment in motor unit recruitment was imported into custom software (LabVIEW, National Instruments, Austin, TX, USA). Such a data set consisted of 4 columns of power data with each column representing the 4 different genetic expressions of slow twitch (Type I and I - IIa) to fast twitch (Type IIa, IIab and Ilb) proportions, where each row represented the sequential summation of contractile power data for each additional recruited motor unit.

For each genetic expression category, the contractile power data for each motor unit row was converted to $totATP_{to}$ based on previously published data for the in-vivo energy transfer of ATP hydrolysis (50 $kJ \cdot mol^{-}$

[1]) and the temperature corrected value of 40% for the cellular efficiency of ATP hydrolysis free energy transfer to muscle contraction (14), as presented in Equation 3.

$$\mathrm{ATP_{to}}\ (\mathrm{mmol \cdot L^{-1}}) = \left(\left(\frac{\text{mechanical power } (\mathrm{J.s^{-1}})}{0.4} \right) \Big/ 50{,}000 \right) \mathrm{X}\ 1{,}000 \qquad \textit{Equation } 3$$

Note that the 40% biological to mechanical energy transfer efficiency value is higher than the known range of whole-body exercise biochemical to physical energy transfer (25 to 30%), which is logical given this range reveals individual (and perhaps exercise mode) differences. Added differences are to be expected given that this model concerned only a single muscle contraction (vastus lateralis; VL), with force data from prior research obtained from direct force measures from single muscle fibres of the VL (5,6), and the 40% efficiency of ATP hydrolysis to mechanical energy value being obtained from recent research of skeletal muscle fibre in-vivo ATP energy transfer (14). As a single muscle contraction does not have the inefficiencies of multiple prime mover muscles, the involvement of postural support muscles, and biological energy expenditure unrelated to the contractile function of the single muscle of interest (vastus lateralis) (e.g. the VO_2 of ventilation), a higher biological to mechanical energy transfer efficiency is to be expected.

The calculation of the $\mathrm{totATP_{to}}$ for specific muscle fibres was completed by the simple division of a given motor unit ($\mathrm{fibres.unit^{-1}}$) by the known number of muscle fibres. The subsequent calculations resulted in motor unit by motor unit increments in summed $\mathrm{totATP_{to}}$ ($\mathrm{mmol \cdot L^{-1}}$). These data sets (1 for each genetic motor unit proportion expression category) were saved as .txt files for subsequent use in further model programming.

It is important to understand that the proportionality of the motor unit categories was based on proportional representation for the 2 categories of slow twitch motor units (I and I - IIa) to being 70 and 30%, respectively; for the 3 categories of fast twitch motor units (IIa, IIa - IIb and IIb) the proportionality was 30, 20, 50%, respectively. There were minor variations to these proportionalities based upon constraints imposed by the model for the total number of muscle fibres of the VL and the ±15% variability in the size ($\mathrm{fibres \cdot unit^{-1}}$) of the motor units. An example of this subcategory motor unit distribution for the 40 - 60 ST - FT motor unit proportionality is presented in Table 1. See Mulligan et al. (13) for further details of the model used to calculate the contractile power of the VL across the different genetic expressions of motor unit proportionality.

Table 1. Subcategory motor unit distribution for the 40 - 60 ST - FT motor unit proportionality.

		Mean Fibre #/unit	Unit #s	Total Fibres	% Total	% Type	Total Fibres	Total %
ST	I	100	1136	113600	28	69.78	162800	
	I - IIa	120	412	49200	12	30.22		40.30
FT	IIa	140	542	75580	19	31.34	241180	
	IIa - IIb	160	279	44640	11	18.51		
	IIb	180	672	120960	30	50.15		59.70
	SUM		3041	403980	100			100

Modelling Motor Unit ATP_{to} During Repeated Muscle Contractions

A new custom program was developed for computing the increase in summed $totATP_{to}$ for contractions of the VL at frequencies of 0.5, 1, 1.5, 2 and 2.5 Hz for fractional motor unit recruitment for increments of 0.05 spanning 0.05 to 0.9. All contraction conditions were for a total duration of 3 min. Data was retrieved for the number of motor units recruited and the summed $totATP_{to}$. To identify the involvement of specific muscle fibre and motor unit types, added software was programmed to convert the row-by-row summed data to individual motor unit-specific data. Based on the known motor unit category from the motor unit size data, $totATP_{to}$ values for contributions of specific motor unit category recruitment were able to be retrieved for all the aforementioned skeletal muscle motor unit and contraction (Hz, fractional recruitment and genetic expression) conditions.

Results

To better inform the presentation and interpretation of the results, Table 2 presents the resulting data for the original model of the VL for the fibre numbers of each motor unit of each category, and for each of the 4 genetic expressions of motor unit proportions. Detailed data of the motor unit features of the model can be found in Mulligan et al. (13).

Table 2. Data for the different motor unit numbers for the different genetic expressions of motor unit proportions used in the model.

	Type	Mean Fibre Number·unit^{-1}	Unit Number	Total Fibres	% Total	% Type	Total Fibres	Total %
80 - 20%								
ST	I	100	2270	227562	56	69.79	326082	
	I - IIa	120	821	98520	24	30.21		80.33
FT	IIa	140	170	24102	6	30.19	79829	
	IIab	160	95	15040	4	18.84		
	IIb	180	226	40687	10	50.97		19.67
	SUM		**3582**	**405911**	**100**			**100**
60 - 40%								
ST	I	100	1716	172025	42	70.60	243665	60.10
	I - IIa	120	597	71640	18	29.40		
FT	IIa	140	359	50300	12	31.10	161760	39.90
	IIab	160	151	24160	6	14.94		
	IIb	180	485	87300	22	53.97		
	SUM		**3308**	**405425**	**100**			**100**
40 - 60%								
ST	I	100	1136	113600	28	69.78	162800	40.30
	I - IIa	120	412	49200	12	30.22		
FT	IIa	140	542	75580	19	31.34	241180	59.70
	IIab	160	279	44640	11	18.51		
	IIb	180	672	120960	30	50.15		
	SUM		**3041**	**403980**	**100**			**100**
20 - 80%								
ST	I	100	562	56200	14	69.04	81400	20.15
	I - IIa	120	210	25200	6	30.96		
FT	IIa	140	680	95200	24	29.51	322600	79.85
	IIab	160	375	60000	15	18.60		
	IIb	180	930	167400	41	51.89		
	SUM		**2757**	**404000**	**100**	**69.04**		**100**

Even though muscles contract by the recruitment of motor units, the new $totATP_{to}$ addition to the model also allowed the calculation of the $totATP_{to}$ for single muscle fibres of the different fibre type categories, as well as the motor unit $totATP_{to}$ across the different motor unit sizes (fibres·unit^{-1}) and fibre type categories. This data is presented in Table 3.

Table 3. Data for the $totATP_{to}$ of single muscle fibres and different motor unit sizes for each of the fibre type categories.

Motor Unit Type		Fibre	Motor Unit	
		$totATP_{to}$*	**$totATP_{to}$^**	**Fibre #**
ST	**I**	0.3293	0.0280	85
	I - IIa	0.4702	0.0480	102
FT	**IIa**	0.9160	0.1090	119
	IIab	1.0744	0.1483	138
	IIb	1.4000	0.2142	153

***$totATP_{to}$ = $\mu M \cdot L^{-1}$; ^$totATP_{to}$ = $mM \cdot L^{-1}$; Note, fibre differences are due to force differences between fibre types; # = predetermined average motor unit sizes within the model.**

The model directly computed the cumulative increase in muscle $totATP_{to}$ for each additional motor unit that was recruited. When assuming the range from zero to total motor unit recruitment the resulting increases in $totATP_{to}$ were non-linear as presented in Figure 2. Note the exponential increase in $totATP_{to}$ with increasing motor unit recruitment for each of the 4 motor unit expression categories, which as expected, revealed more rapid increases in $totATP_{to}$ with increasing recruitment for the VL with greater fast twitch motor unit expression. For this model, such changes were not based on cellular differences in metabolic efficiency between muscle fibre types (assumed to be constant and equal across motor unit types), but by the increasing size ($fibres \cdot unit^{-1}$) with increasing fast twitch motor unit expression (see Discussion).

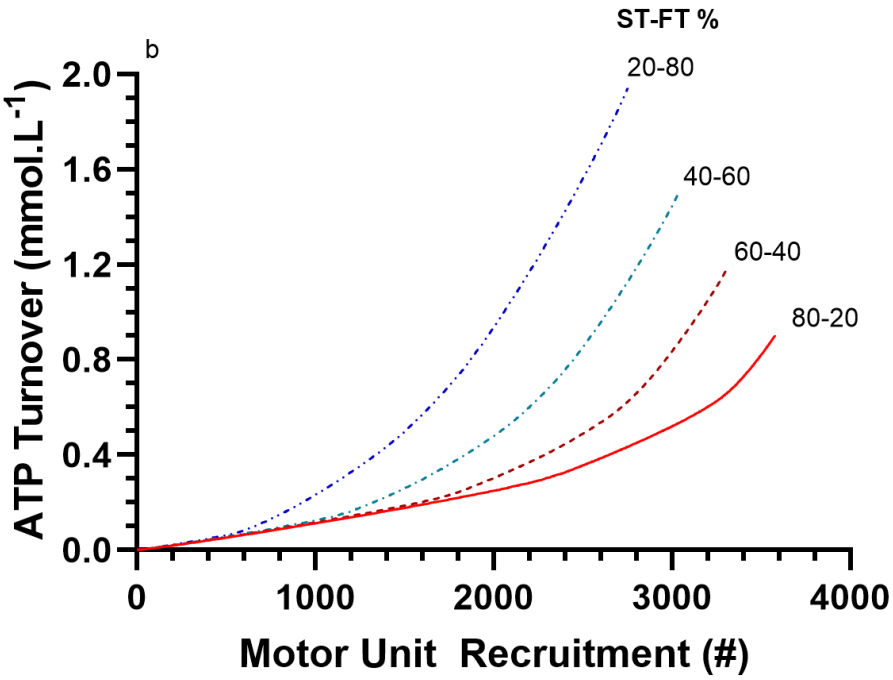

Figure 2. The increase in cumulative totATP$_{to}$ for incremental motor unit recruitment spanning 0 - 100% of the motor unit pool for 4 different categories of slow twitch to fast twitch (ST - FT%) motor unit expression.

As voluntary movement, such as muscle contractions during exercise, involves repeated muscle contractions of varied intensity and therefore motor unit recruitment, calculations were also completed for 3 min of VL contractions at different frequencies of contraction and percentage of motor unit recruitment. The resulting data for totATP$_{to}$ are presented in Figure 3a-d, and totATP$_{to}$ data for the 2 extremes of contraction frequency and motor unit recruitment across the 4 genetic expression categories for the 3 min repeated contractions conditions are presented in Table 4.

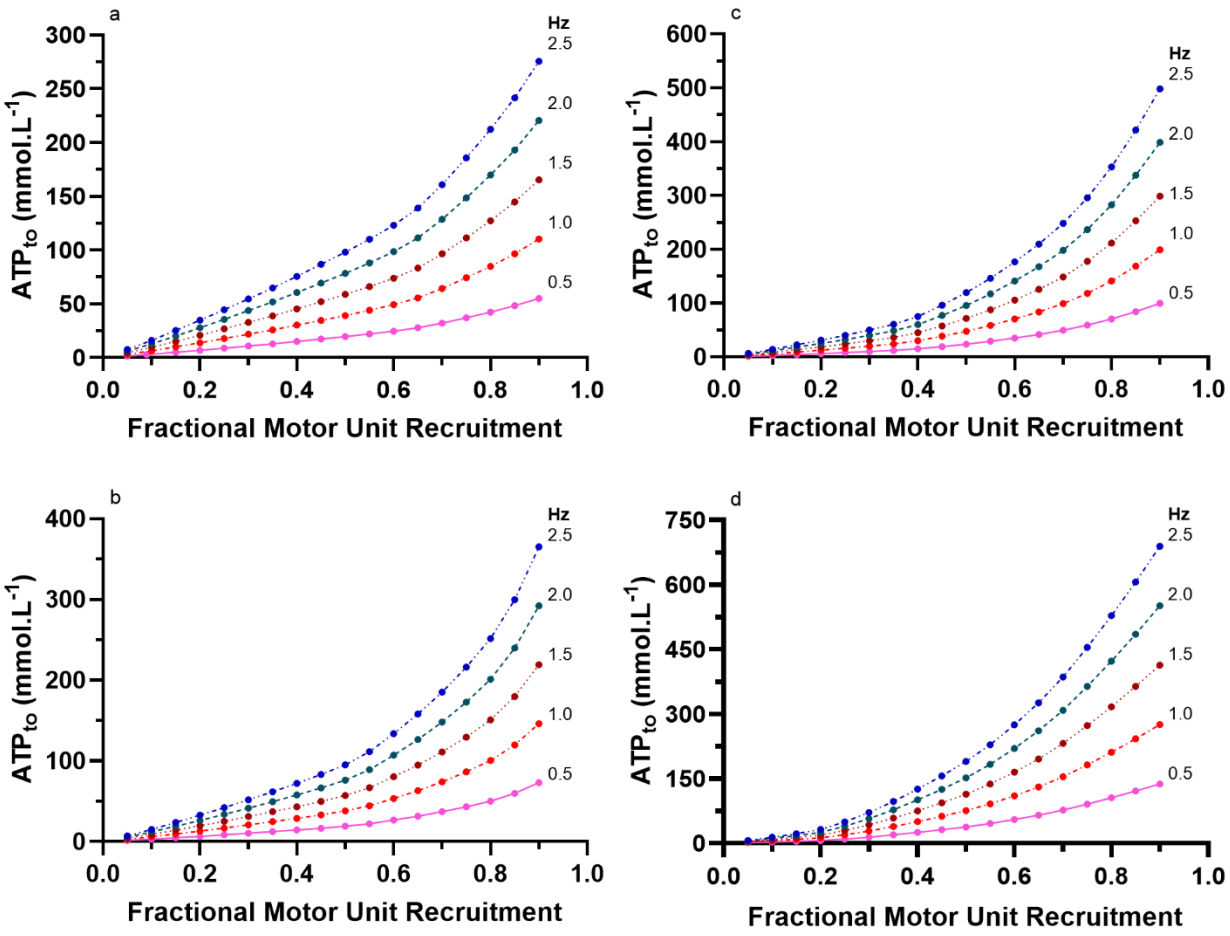

Figure 3. The cumulative totATP$_{to}$ for increasing fractional (0.05 to 0.9) motor unit recruitment for 3 min of different frequencies of contraction of the vastus lateralis (VL) for a) 80 - 20%, b) 60 - 40%, c) 40 - 60% and d) 20 - 80% ST to FT expression.

Table 4. Summary data for condition extremes for totATP$_{to}$ (mmol·L^{-1})

Recruitment*	**0.05**	**0.9**	**0.05**	**0.9**
Frequency (Hz)	**0.5**		**2.5**	
80 - 20	1.5	55.1	7.5	275.7
60 - 40	1.4	73.0	7.1	365.0
40 - 60	1.3	99.6	6.7	498.2
20 - 80	1.3	137.8	6.5	689.0

* = fractional

Data of the $totATP_{to}$ for specific muscle fibre types for 90% recruitment across 5 different rates of contractions for the 3 min conditions are presented in Table 5. For the 80 - 20% ST - FT expression, 90% recruitment required a subset of the Type IIab motor units and did not involve any Type IIb motor units.

Table 5. Computed data for motor unit $totATP_{to}$ ($mmol \cdot L^{-1}$) for the involvement of the 5 different motor unit types for contraction conditions of 0.5, 1.0, 1.5, 2.0 and 2.5 Hz for 90% motor unit recruitment.

ST - FT	Contraction Frequencies (Hz)				
Category	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**
80 - 20					
I	16.7	31.3	47.6	66.6	82.0
I - IIa	18.9	36.4	55.6	75.0	93.7
IIa	17.1	33.8	51.8	68.2	85.3
IIab	2.5	4.8	7.4	9.5	12.0
IIb	0	0	0	0	0
60 - 40					
I	12.6	23.6	36.0	49.5	61.9
I - IIa	14.0	27.0	41.3	55.6	69.5
IIa	14.4	28.3	43.5	57.0	71.4
IIab	11.7	22.8	35.1	45.5	57.4
IIb	20.5	40.9	61.0	80.9	102.1
40 - 60					
I	8.3	15.6	23. 8	32.7	40.9
I - IIa	9.4	18.2	27.8	37.4	46.8
IIa	13.3	26.1	40.1	52.4	65.7
IIab	18.8	36.7	56.3	73.0	92.2
IIb	50.4	100.3	149.2	198. 3	250.1
20 - 80					
I	4.1	7.7	11.7	16.1	20.2
I - IIa	4.7	9.1	14.0	18.8	23.5
IIa	11.5	22.4	34.5	44.9	56.5
IIab	25.0	49.1	75.2	97.5	123.2
IIb	93.4	185.8	276.3	367.4	463.3

To improve the visual comparison of the $totATP_{to}$ between the different motor units, stack plot column graphs were completed and presented in Figure 4 for $totATP_{to}$ contributions from the different motor unit categories for 90 % recruitment and 2.5 Hz contractions for 3 min.

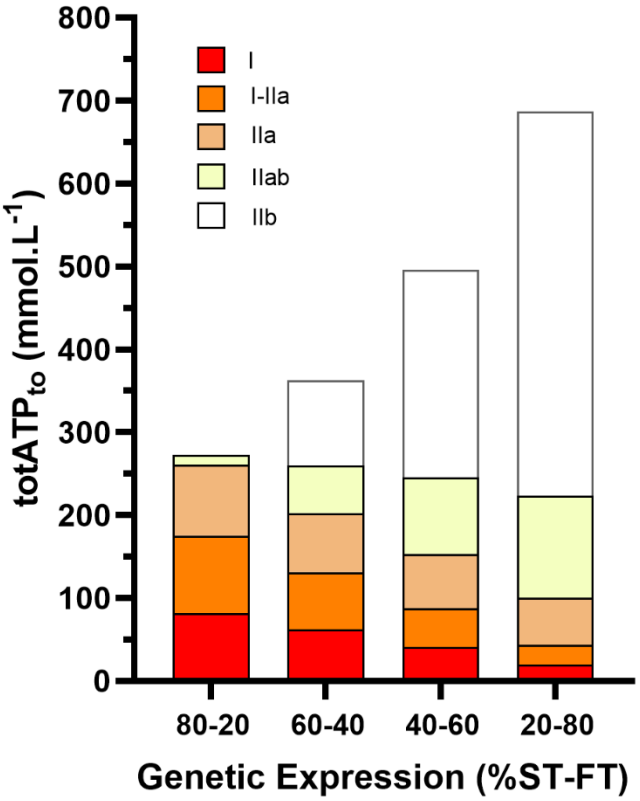

Figure 4. Differences in $totATP_{to}$ across different genetic expressions and motor unit types for 3 min of repeated contractions at 2.5 Hz and 90% motor unit recruitment.

Discussion

A model for the motor unit by motor unit increments in skeletal muscle contractile power was used as the source of motor unit increments in muscle contractile power (13). The temperature corrected cellular efficiency of ATP energy transfer to mechanical power generation (40%) and the in-vivo cellular free energy release of hydrolysis (50 $kJ \cdot M^{-1}$) (14) were used to account for the conversion of contractile mechanical power (1 Watt = 1 $J \cdot s^{-1}$) to cellular metabolism expression of free energy release (Equation 1), which was then re-expressed to $totATP_{to}$ ($mmol \cdot L^{-1}$). The model was then applied to 3 min of repeated contractile conditions spanning 0.5 to 2.5 Hz and 5 to 90% motor unit recruitment across 4 genetic expressions of motor unit proportions.

Single fibre $totATP_{to}$ for Type I vs Type IIb equated to 0.3293 to 1.40 $\mu M \cdot L^{-1}$. The $totATP_{to}$ for the VL ranged from 0.0280 to 0.2142 $mM \cdot L^{-1}$ for Type I vs Type IIb, respectively. Data for 90% motor unit recruitment during 3 min of repeated contractions at 2.0 Hz revealed $totATP_{to}$ of 220.55 vs 551.16 $mM \cdot L^{-1}$ for genetic expressions of 80 - 20 vs 20 - 80 (ST - FT%), respectively.

The structure of this discussion will transition from the results for the $totATP_{to}$ of single muscle fibres to motor units and then overall ATP_{to} for different proportional motor unit recruitment of repeated contractions at different contraction frequencies. Where suited, results will be compared to prior research data.

Methodological Details

As temperature rises, enzyme activity within muscle cells accelerates, and the cellular biochemical efficiency for free energy transfer also increases (14,5). He et al. (14) compared the results of muscle fibres' force-velocity and power-velocity curves at 12ºC and 20ºC. The temperature increase caused a marked increase in maximum shortening velocity, peak power, $totATP_{to}$ and efficiency for all fibre types. As such, our model was adjusted to account for peak efficiency at 20ºC, which Bottinelli et al. (5) demonstrated to be a temperature above which no further changes in efficiency occurred.

Single Fibre and Motor Unit ATP_{to}

There is a limited amount of research that has quantified single fibre $totATP_{to}$ in humans. He et al. (14) presented results for $totATP_{to}$ under different conditions of contractile power and velocity of isolated single muscle fibres using chemo-mechanical transduction methodology that allowed near direct measurement of the rate of ATP hydrolysis. As this data was not presented for a single contraction condition or over repeated contractions, we are unable to compare this data with the current model. However, they displayed remarkably similar metabolic to contractile power efficiencies across all muscle fibre types. For this reason, the current model adopted the same efficiency value (0.4) and the experimental temperature condition (20ºC) across all fibre type categories as previously explained.

The study by Bottinelli et al. (5) provided valuable insight into single fibre muscle biochemistry. It demonstrated the influence that muscle fibre type had on many of the parameters (such as maximum shortening velocity, maximum power output, and optimal velocity). Notably, there was a significant difference in capacity to produce mechanical work between the 3 fibre types (slow [Type I], fast [Types IIa and IIb] and mixed [Types I - IIa and IIa - IIb]). This is reflected in the current model, which found slower fibre types having less capacity for contractile force, resulting in a lower contribution to cellular $totATP_{to}$. Furthermore, it revealed that efficiency did not increase further at higher temperature conditions. Such

differences were further expanded in the model based on the larger muscle fibres·unit^{-1} in FT motor units based on the Size Principle of motor unit recruitment. While these studies do not provide quantified totATP$_{to}$, meaning we are unable to provide a comparison to the current model, they provide valuable insight into the contractile function difference between muscle fibre types. This leaves whole muscle-based estimations of totATP$_{to}$ to be the only source of prior published data for comparison purposes, presented below.

Comparisons of Total ATP$_{to}$ to Prior Research

Figure 1 presents the prior research that has quantified whole muscle anATP$_{to}$ during intense exercise in humans. Other research has been completed that quantified ATP$_{to}$ for exercise conditions not conducive to maximal capacities for muscle ATP$_{to}$. For example, Bendahan et al. (15), Chasiotis et al. (16), and Katz et al. (17), provided totATP$_{to}$ data equating to 43.5, 89.6, and 62.6 mmol·L^{-1}, respectively. However, as this data was based on relatively low exercise intensities, short durations, and/or a small muscle mass compared to prior research of maximal anATP$_{to}$ (Figure 1), the data are not comparable. Kemp et al. (4) quantified a totATP$_{to}$ of 57 mmol·L^{-1} for the flexor digitorum superficialis during 1 W power output forearm wrist flexion exercise. As this was for a mix of muscle fibre types, a small muscle and exercise that was not performed to failure, there is limited comparison to the current modelled research.

To compare data from prior studies that used different units for ATP$_{to}$, data conversions were needed. Many of the previous research studies reported anATP$_{to}$ in mmol·kg^{-1} dry wt. that required conversion to mmolkg^{-1} wet wt, using the following equation (12,18) (Equation 4).

$$[\text{metabolite}]\ \text{mmol kg}^{-1}\text{wet wt.} = \frac{[\text{metabolite}]\ \text{mmol. kg}^{-1}\ \text{dry wt.}}{4.17} \qquad Equation\ 4$$

For conversion from kg wet wt. to L^{-1} muscle water, the value was further divided by 0.76 (12,18) (Equation 5).

$$[\text{metabolite}]\text{mmolL}^{-1}\ \text{muscle water} = \frac{[\text{metabolite}]\ \text{mmol. kg}^{-1}\ \text{wet wt.}}{0.76} \qquad Equation\ 5$$

The most comparable prior research to the current results of the model were based on estimations of anATP$_{to}$ by Robergs (12), Bangsbo et al. (1), Spriet et al. (3) and Medbo and Tabata (2). Such anATP$_{to}$ data were presented in Figure 1 and were converted to totATP$_{to}$ (aerobic + anaerobic) based on the results of

Chasiotis et al. (16) and Bendahan et al. (15) where anaerobic ATP_{to} represented 48% of total ATP_{to}. As such, Figure 1 has been redrawn and presented as Figure 5 and now includes comparisons to current $totATP_{to}$ results derived from the model.

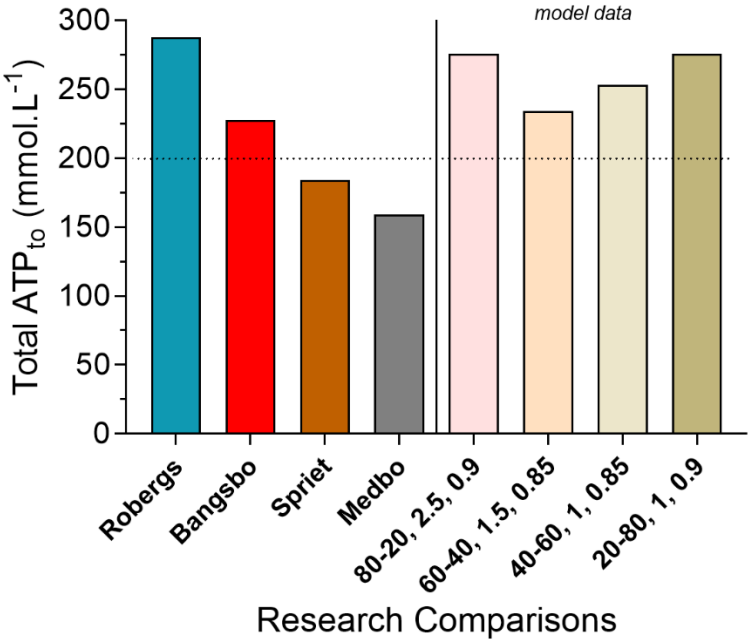

Figure 5. The $totATP_{to}$ (anaerobic and aerobic) data re-computed from Figure 1 as well as from closest data obtained for different genetic expressions, contraction frequencies and fractional recruitment from the current model. For example, the x-axis label of 60 - 40, 1.5, 0.85 refers to 60 - 40 %ST - FT, 1.5 Hz and 0.85 fractional motor unit recruitment.

The current data compare favourably to the modelled data of Robergs (11) and Robergs (12) even though the results were from 2 different computation models. For example, the prior modelled data of $anATP_{to}$ from Robergs (11) was calculated from prior data of muscle metabolite accumulation. The current model was based on $totATP_{to}$ calculations derived from the cellular free energy of ATP hydrolysis and energy transfer efficiency. The results of these methods are logically higher than prior exercise-based muscle biopsy research because the current model did not factor into calculations a decreasing contractile function with increasing contractile failure. Further, this research focussed on just one muscle being the sole source of contractile power. Conversely, whole body exercise, such as cycling, walking or running, distributes muscle contractile power across multiple muscles, thereby lowering the ATP demand for the mechanical power output for any single muscle.

Partitioning ATP_{to} for Aerobic vs. Anaerobic Metabolic Pathways

Many of the aforementioned studies (text and Figure 5) used methodology focussed on calculating the $anATP_{to}$, thereby only revealing a subset of true $totATP_{to}$ (anaerobic and aerobic). Krustrup et al. (19)

addressed this issue by conducting a study examining muscle heat generation, oxygen consumption and anaerobic energy turnover during repeated high-intensity exercise. In this study, participants performed 3 consecutive 3 min bouts of intense one-legged knee extensor exercise to calculate the $totATP_{to}$ based on measurements of heat production. This yielded a breakdown of 23% anaerobic and 77% aerobic contributions to $totATP_{to}$. This contrasts with the findings of Bendahan et al. (1), who reported 52% anaerobic and 48% aerobic contributions.

Based on the data from Krustrup et al. (19) and the calculated 23% (of total) anaerobic ATP_{to}, the anaerobic component of their total ATP_{to} was determined to be 170 $mmol \cdot L^{-1}$. According to our calculations, if the VL was contracting at 90% recruitment, a 60-40% proportionality would yield remarkably similar results, with an approximate ATP_{to} of 190 $mmol \cdot L^{-1}$ (I - 70.6%, I – IIa - 29.4%, IIa - 31.1%, IIab - 14.94%, and IIb - 53.97%). To compare the total metabolic energy turnover (37.83 kJ), given that the mass of the exercised muscle and the % of motor unit recruitment are unknown in the research by Krustrup et al. (19), it can be assumed that both factors would equate to a similar muscle motor unit involvement to the single muscle VL model of this research. Consequently, using a constant mechanical efficiency of 50% from He et al. (14), the total ATP_{to} for Krustrup et al. (19) was estimated to be 760 $mmol \cdot L^{-1}$. This finding closely aligns with our total ATP_{to} (689 $mmol \cdot L^{-1}$) for 20 - 80 at 90% recruitment, demonstrating that the data is not only valid but that motor unit type and therefore fibre type specific. For example, for the VL genetic motor unit proportion expression of 80-20% (ST-FT) at 2.5Hz and 90% recruitment yielded a total ATP_{to} of 276 $mmol \cdot L^{-1}$. There is also a need to recognize the importance of the variability in aerobic:anaerobic ATP_{to} with changes in muscle ST-FT motor unit proportionality. For example, logically the ratio of the 2 contributions would be highest for the 80 - 20 %ST-FT condition, and lowest for the 20-80 %ST-FT condition.

Limitations

This model provided valuable insight and a framework for quantifying $totATP_{to}$ in skeletal muscle; however, several limitations need to be acknowledged. Firstly, this model primarily focussed on 5 distinct muscle fibre types. Research suggests that there could be considerably more than 5 distinct muscle fibre types, each with unique motor unit recruitment and metabolic characteristics (20). Therefore, the involvement of added motor unit types could improve future versions of the modelling of motor unit recruitment.

This model has a limited set of genetic expressions to estimate $totATP_{to}$. A more comprehensive exploration of genetic expressions may yield a more nuanced understanding. Additionally, our model assumed a

constant contractile performance and no changing efficiency for each contraction across the various frequency conditions and the different muscle fibre types. Refinement of the model with the capacity to overcome these limitations will contribute to a more comprehensive and accurate representation of $totATP_{to}$ dynamics in skeletal muscle, advancing understanding of this physiological phenomenon. Regardless, as mentioned above, the results from the model conformed remarkably close to results from prior research.

This model assumed the VL was able to perform each contraction to the same force, power and $totATP_{to}$, and did not include the reduced ATP_{to} that would occur with increasing contractile failure. This would have resulted in a higher $totATP_{to}$. Future models accounting for this would be beneficial.

Recommendations For Future Research

To advance the understanding of muscle energetics, future research should investigate motor unit-specific contributions to $totATP_{to}$ and its aerobic and anaerobic components. Historically, studies have relied heavily on mixed muscle sampling, limiting the ability to discern the distinct metabolic dynamics of motor units. Utilising animal models with muscles predominantly composed of single motor unit type expressions (e.g., dog or rat hindlimbs) can offer a more precise assessment of motor unit specific $totATP_{to}$, which can help increase the accuracy of such models.

As this model is the first of its kind, it warrants critical evaluation and enhancement. Continual refinement of this model, such as incorporating elements of summation and/or isometric contractions, and the addition of multiple muscles with different contractile power curves for a movement action will significantly improve its accuracy and comprehensiveness.

Conclusions

This research has addressed a critical gap in the existing literature by developing a comprehensive model for quantifying $totATP_{to}$ in skeletal muscle at the muscle fibre level and motor unit level, while also accounting for metabolic differences between the divergent muscle fibre types. The scarcity of research in this area highlights the need for further investigations. The results provide valuable insight into $totATP_{to}$, from single motor units to overall $totATP_{to}$ under varying conditions of motor unit recruitment and contraction frequencies. These findings are particularly relevant when compared to prior studies and provide a foundation for future research.

It is recommended that future investigations focus on motor unit-specific contributions to $totATP_{to}$, utilising animal models to better understand the metabolic dynamics of motor units. Additionally, the continual

refinement of the current model, including the incorporation of elements of summation and isometric contractions, will contribute to a more robust tool for quantifying $totATP_{to}$ during repeated contractions of skeletal muscles.

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